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TECHNICAL MANUSCRIPT 288

STIMULATION OF RNA SYNTHESIS, PROTEIN SYNTHESIS, AND ABSCISSION BY ETHYLENE

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UNITED STATES ARMY
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STIMULATION OF RNA SYNTHESIS, PROTEIN SYNTHESIS, AND ABSCISSION BY ETHYLENE

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ABSTRACT

Ethylene stimulated RNA and protein synthesis in bean (Phaseolus vulgaris L. var. Red Kidney) abscission zone explants prior to abscission. The effect of ethylene on RNA synthesis and abscission was blocked by actinomycin D. Carbon dioxide, which inhibits the effect of ethylene on abscission, also inhibited the influence of ethylene on protein syntheses. An aging period appears to be essential before bean explants respond to ethylene. Kinetins, which retard senescence, will also block the action of ethyiene. Stimulation of protein synthesis by ethylene occurred only in receptive or senescent explants. Treatment of juvenile explants with ethylene, which has no effect on abscission, also has no effect on protein synthesis. Evidence in favor of a hormonal role for ethylene during abscission is discussed.

I. INTRODUCTION

In an earlier paper we[1] showed that the protein synthesis inhibitors actinomycin D and cycloheximide would block abscission, and that during the course of ethylene-enhanced abscission C^{14} L-leucine was incorporated into a trichloroacetic acid precipitable fraction of tissue homogenates from the separation zone of bean (Phaseolus vulgaris L. var. Red Kidney) and cotton (Gossypium hirsutum var. Acala 4-42) explants. Protein synthesis in the stem and in the nodal tissue surrounding the separation zone was not influenced by the ethylene treatment. These results supported the view that ethylene, like other hormones,[2] was capable of acting as an effector.

In this paper we will show that the stimulation of RNA synthesis and protein synthesis is analogous to that reported for other hormone systems.

II. MATERIALS AND METHODS

Methods used to grow and prepare explants as well as to measure ethylene in the surrounding gas phase have been described earlier.[3,4]

A. PREPARATION OF RNA

The phenol method of RNA extraction is a modification of the methods used by Ingle et al.[5] and Cherry and Huystee.[6] One and one-half grams of tissue (15 explants) were homogenized in 6.5 ml phenol (washed and saturated with 0.01 M Tris buffer, pH 7.5), 0.2 ml 25% SLS (sodium lauryl sulfate), 0.30 ml bentonite (150 mg/ml), and 3.5 ml 0.01 M Tris buffer (pH 7.5) with a VirTis 23 homogenizer for one minute at high speed, followed by stirring for 4 minutes. After centrifugation (10,000 x g, 10 minutes) the aqueous phase was removed and further deproteinized by one 3-minute extraction with an equal volume of phenol. The final aqueous phase was made 0.15 M with respect to potassium acetate, and the nucleic acid was precipitated by adding ethanol to a final concentration of 70%. After standing at -15 C for at least 2 hours, the nucleic acid was removed by centrifugation (20,000 x g, 10 minutes) and dissolved in 1.25 ml of 0.05 M phosphate buffer, pH 6.7. This solution was dialyzed against 0.05 M phosphate buffer, pH 6.7, for 48 to 72 hours (two changes of 5 liters each). Samples were taken for optical density (O.D.) readings at 260 mμ, and samples were plated for radioactivity.

The perchloric acid method of RNA extraction was modified after Key and Shannon.[7] One and one-half grams of tissue (10 to 15 explants) were homogenized in 2 ml 0.01 M Tris buffer, pH 7.5, in a ground glass homogenizing flask with a ground glass pestle for 1 to 2 minutes. The sides of the homogenizing flask were washed down with 3 ml of 0.01 M Tris buffer and the resultant suspension was filtered through glass wool. Samples (3.5 or 4.0 ml) were taken immediately after filtration through glass wool, made to 0.2 N with respect to $HClO_4$, thoroughly mixed, and centrifuged at 1,000 x g for 10 minutes. The pellets were then suspended and washed twice with 0.2 N $HClO_4$ and centrifuged at 1,000 x g for 10 minutes. The resulting pellets were then suspended and washed twice in methanol containing 0.05 M formic acid and centrifuged at 2,000 x g for 10 minutes. All above steps, including centrifugation, were carried out at 2 to 4 C. The washed pellets were twice extracted at 37 C for 30 minutes in a 2:2:1 mixture of ethanol:ether:chloroform to remove lipids and centrifuged at 3,000 x g for 10 minutes. RNA was hydrolyzed in 0.3 N KOH for 18 hours at 37 C. After chilling, $HClO_4$ was added to a final concentration of about 0.3 N, followed by centrifugation at 2,000 x g for 10 minutes to remove the $KClO_4$ precipitate, protein, and DNA. Absorbance of samples of RNA was measured at 260 and 290 mμ with a spectrophotometer. After determining the RNA, each sample was neutralized with KOH to pH 4.5 to 5.5. After chilling, the $KClO_4$ precipitate was removed by centrifugation and samples were plated, dried, and counted for determination of P^{32} incorporation into RNA.

B. PROTEIN EXTRACTION METHODS

Proteins were extracted by a modified Peterson and Greenberg[8] method. Tissue was homogenized in ground glass homogenizers with 1 ml 0.01 M Tris buffer, pH 7.5, containing 0.2 mg C^{12} L-leucine per ml. After two minutes' homogenization, 3 ml of Tris buffer were added and the resultant suspension was poured into centrifuge tubes. A small sample of the homogenate was taken for extraction in hot 80% ethanol to determine the amount of ethanol-soluble C^{14} L-leucine. The amount of ethanol-soluble C^{14} L-leucine was essentially the same (±10%) from ethylene-treated and control explants. The remaining homogenate was centrifuged at 775 x g for 10 minutes. The sediment was washed with distilled water and 0.01 N NaOH. The supernatant fractions were combined with the original supernatant. The proteins were precipitated by adding two volumes of 20% trichloroacetic acid (TCA) and purified by successive washings with 5% TCA (4 times), ethanol (once), 3:1 ethanol-ether, 3 minutes at 60 C (4 times), and ether (once). During the second 5% TCA wash, the suspension was heated at 90 C for 15 minutes. The final protein pellet was suspended in 1 N NaOH and a sample was taken and diluted to 0.1 N NaOH for protein analysis by the Lowry method.[9] A less exhaustive but simpler method of protein extraction that gave identical results is based on a method described by Key;[10] it has been published earlier.[1]

C. INCUBATION OF EXPLANTS

Six milliliters of 1.5% agar were poured into 43 ± 2-ml gas-collection bottles (5 cm in diameter and 2.5 cm high) and 10 explants were inserted in the agar so that 3 mm of the explant were submerged. The bottles were fitted with a neck in which a 25-mm-diameter rubber vaccine cap could be placed. Where required, ethylene was injected into the bottles through the vaccine cap to give the specified ethylene concentration. The gas-collection bottles were cut horizontally 1.25 cm from the bottom to facilitate manipulation of the agar containing radioactive compounds and explants. To make the bottles gastight, silicone grease was applied to the ground surfaces and the bottle halves were held together with adhesive tape. Control explants were aerated by opening and resealing the gas-collection bottles. The ethylene concentration in the gas phase was monitored by gas chromatography and in the aerated bottles the concentration was not allowed to exceed 0.1 nl ethylene per ml (0.1 ppm) during the experiments. Previous experiments by Rubinstein and Addicott[4] indicate that this amount of ethylene does not accelerate abscission over that in aerated controls. The bottles were incubated at 25 C under 400 ft-c of continuous fluorescent light (General Electric type 30T8-ww, warm white).

D. APPLICATION OF P^{32} AND C^{14} L-LEUCINE TO ABSCISSION-ZONE EXPLANTS

Radioactive orthophosphate in neutralized solution, carrier-free, was incorporated in 1.5% agar disks (1.4 mm x 3 mm-diameter) that contained approximately 3 μc of P^{32}. The amount of C^{14} L-leucine incorporated in these agar blocks (0.01 ml of 2.8×10^{-5} M except that shown in Table 4, 10^{-4}) was not high enough to act as an abscission stimulant.[4] Concentrations greater than 10^{-3} M are required for this effect.

The disks of C^{14} L-leucine were saved at the end of an experiment, placed in planchets, and melted by adding 1 ml of water and heating. This treatment spread the disks (usually 10 per replicate treatment) evenly over the bottom of the planchet. The planchets were then counted to check whether ethylene had any effect on C^{14} L-leucine uptake into the tissue; no effect has been observed.

All tissue samples were immediately frozen after harvesting and thawed just before homogenization.

III. RESULTS

A. PREPARATION OF SENESCENT EXPLANTS

Earlier workers[11,12] recognize two distinct phases or stages during the abscission of bean explants. Yamaguchi[12] reported that during phase I, bean explants were insensitive toward ethylene; during phase II, however, the gas stimulated abscission. Later, Rubinstein and Leopold[11] reported that abscission of bean explants could be characterized by two stages, stage I in which auxins inhibit abscission, and II in which auxins stimulated abscission. Abeles and Rubinstein[3] confirmed Yamaguchi's original observations and found that the stimulation of abscission by auxin during stage II was due to a stimulation of ethylene evolution from the explants by auxin.

We propose to substitute the self-explanatory terms "juvenile" for phase I (stage I) and "senescent" for phase II (stage II) and thereby avoid the use of terminology that requires some prior knowledge of this area of plant physiology. Since insensitivity toward ethylene for 12 to 24 hours, depending on the temperature,[12] seems to be peculiar to bean abscission-zone explants (as opposed to explants from cotton and Coleus), this terminology is limited to bean explants at this time.

The transition from juvenility to senescence can be characterized by a mobilization of materials such as chlorophyll, protein, RNA, and phosphate out of the pulvinus and into the petiole part of the explant.[13] Cytokinins, which are known to delay senescence of leaf tissue, inhibit these mobilization phenomena.*

Kinetin not only delays senescence of explants but also delays abscission.[14] Osborne and Moss[14] found that application of kinetin close to the separation zone of bean explants delayed abscission; an application to the pulvinal stump accelerated abscission. We have found (Fig. 1) that the more mobile kinetins such as N^6-benzyladenine and SD 8339 [(6-benzylamino-9-2-(tetrahydropyranyl-9H-purine)] retard abscission, regardless of their point of application, and kinetin (6-furfurylamino purine) showed no effect at any concentration used. In other experiments not presented here, kinetin stimulated abscission over that of the controls. This abscission is probably due to a stimulation of ethylene production from explant tissue as a result of the kinetin treatment. These observations emphasize the need to monitor ethylene production from plant tissues during experimental manipulation so as to avoid overlooking potential complications in interpretation arising from enhanced ethylene levels around ethylene-sensitive tissue. Of the kinetins tested, SD 8339 was observed to have practically no effect on ethylene production from explant tissue. Essentially similar results were obtained using explants from cotton and Coleus. In these experiments, preparation of cotton and Coleus explants followed the method of Carns et al.[15] and Gorter[16] respectively.

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* P.C. Scott, personal communication.

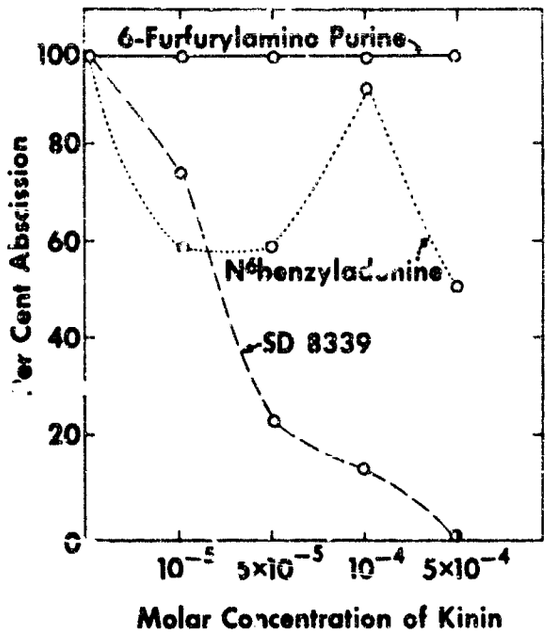

Figure 1. Effect of Cytokinins on Bean Explant Abscission. Explants were inserted petiole end down in 1.5% agar containing the various cytokinins. Abscission read 24 hours after the start of the experiment.

The fact that kinetin (SD 8339)-treated explants (Table 1) fail to abscise when exposed to ethylene is additional support for the hypothesis that only senescent tissue is ethylene-sensitive. Except for those shown in part A of Fig 5, only senescent explants were used in the experiments reported in this paper. After the explants were cut from the primary bean leaf, they were stored by sticking them in 3 mm of 1.5% agar in petri dishes for 22 ± 2 hours and placing the petri dishes in the incubation chamber (described above).

TABLE 1. EFFECT OF 6-BENZYLAMINO-9-2-TETRAHYDROPYRANYL-9H-PURINE (SD 8339) ON EXPLANT[a] ABSCISSION

Treatment	% Abscission
Control	60
4 ppm ethylene	100
SD 8339	0
4 ppm ethylene + SD 8339	0

a. Explants were aged for 22 hours at 25 C with their pulvinal ends placed in either plain agar or agar containing 5×10^{-4} M SD 8339. Ethylene was added at 22 hours and abscission measured 8 hours later.

B. INHIBITION OF ABSCISSION BY ACTINOMYCIN D AND CYCLOHEXIMIDE

Both actinomycin D and cycloheximide are known to be potent inhibitors of protein synthesis. Actinomycin D is thought to block DNA-dependent RNA synthesis.[17] However, other effects of this compound, such as an inhibition of respiration and glycolysis of human leukemic leukocytes, have been reported.[18] Cycloheximide is thought to act later in the sequence of events leading to protein synthesis. Ennis and Lubin[19] have shown that cycloheximide specifically inhibits transfer of amino acids from soluble RNA to polypeptide in extracts of rat liver. However, the action of this compound is not universal, as this drug had no effect on protein synthesis in extracts of Escherichia coli. Since actinomycin D inhibits at an earlier point than cycloheximide in the sequence of events leading to protein synthesis, a time course curve showing the effect of these inhibitors on ethylene-treated explants should show that the inhibiting effect of cycloheximide lasts longer than that of actinomycin D.

Actinomycin D and cycloheximide were injected into the explant in 1 µl of solution with a microliter syringe by sticking the needle up through the center of the petiole tissue to a depth of about 5 mm, at which point the firmer pulvinal tissue resists further movement of the needle. After the needle was withdrawn, all of the solution remained within the cavity of the petiole tissue. The first injection was made immediately prior to the addition of the ethylene. Two, four, and six hours later a set of bottles was opened, inhibitor was injected into the explants, and the bottles were resealed and the ethylene reinjected. Abscission was measured 9 hours after the start of the experiment. As shown by the representative experiment in Fig 2, the ability of the actinomycin D to delay abscission decreases after the addition of ethylene, but cycloheximide retains its effectiveness for about 4 hours. Experiments performed on two other occasions gave essentially similar results.

Data in Table 2 show that the action of actinomycin D in explant tissue is due to an inhibition of RNA synthesis. Explants were injected with actinomycin D (described above) before exposure to ethylene. The agar blocks containing P^{32} were placed on top of the explants, and the RNA was extracted and measured 4 hours later. Data in Table 2 show that actinomycin D inhibits RNA synthesis of both control and ethylene-treated explants.

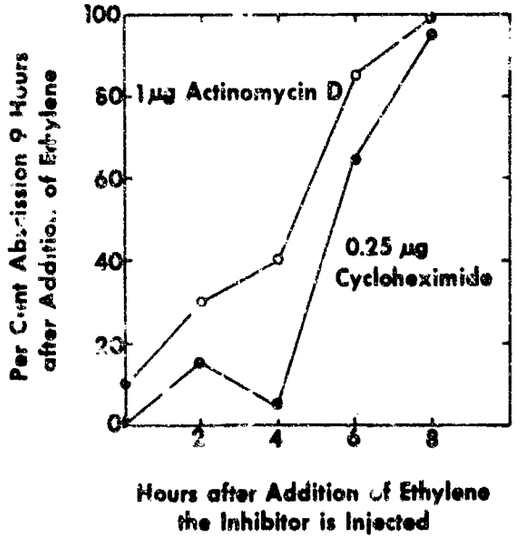

Figure 2. Effect of Actinomycin D and Cycloheximide on Abscission of Senescent Explants.

TABLE 2. EFFECT OF 1 μg ACTINOMYCIN D ON P^{32} INCORPORATION INTO BEAN EXPLANT[a] RNA

Treatment	% Abscission after 29 hours	CPM[b]/mg RNA
Control	23	44,000 ± 7,000
2 ppm ethylene	68	102,000 ± 7,000
Actinomycin D	8	27,000 ± 5,000
2 ppm ethylene + actinomycin D	8	34,000 ± 6,000

a. Explants were aged 22 hours at 25 C and then injected through the petiole with either 1 μl of water or 1 μl of actinomycin D. Two hours later the agar blocks containing P^{32} were put on the pulvinus and the gas collection bottles were sealed. The explants were harvested 5 hours later. RNA was extracted and purified by the perchloric acid method.

b. Counts per minute.

C. EFFECT OF X-IRRADIATION

Another way of implicating an interaction between ethylene and the nucleic acids of the cell in abscission is to alter the priming activity of the DNA with X-irradiation. Grosch[20] discussed recent evidence that nucleic acid polymers are more sensitive to X-irradiation than proteins, and Harrington[21] has shown that the ability of *E. coli* DNA to prime for RNA- and DNA-polymerases was depressed by doses of 1,000 r and less.

If an intact nucleus is required for ethylene action, then X-irradiation prior to addition of ethylene to susceptible explants should block or retard the abscission-stimulating effect of ethylene. If the nucleus is exposed to X-rays after ethylene has had a chance to initiate polypeptide formation, the X-rays should have little or no effect on abscission. Senescent explants were exposed to a General Electric Maxitron 1,000 for 30 minutes at a distance of 10 cm, giving a total dose of 260,000 r. Explants were treated immediately prior to the addition of ethylene and then 2.5 and 5 hours later. Abscission was measured 8 hours after the start of the experiment. As shown in Figure 3, an exposure of 260,000 r prior to an inductive ethylene treatment resulted in an inhibition of abscission. The same treatment 2.5 hours later had only a residual effect, and a 5-hour treatment gave results similar to that of untreated controls.

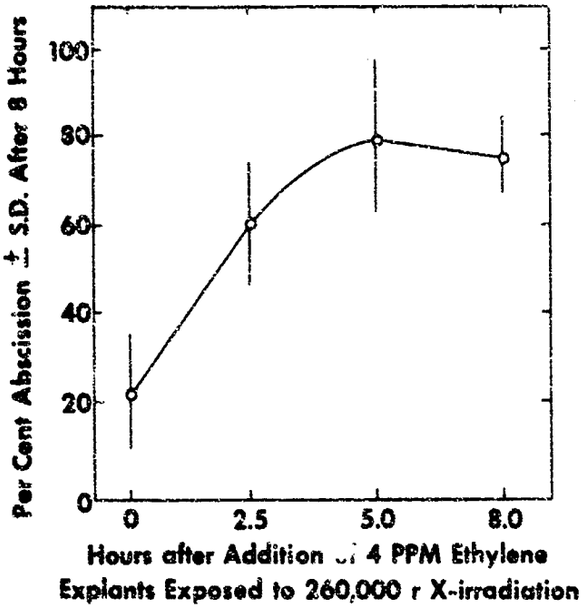

Figure 3. Effect of X-Irradiation on Abscission of Senescent Explants Exposed to 4 PPM Ethylene.

D. ENHANCEMENT OF RNA AND PROTEIN SYNTHESIS BY ETHYLENE

The use of inhibitors and X-irradiation can only give indirect support to the idea that ethylene is capable of acting as an inducer in the abscission process. A more direct approach is to show that ethylene is capable of stimulating RNA synthesis in receptive tissue, followed by a stimulation in protein synthesis.

In this experiment, agar blocks of P^{32} and C^{14} L-leucine were placed on top of senescent explants, and the explants were incubated in either air or an atmosphere containing 4 ppm ethylene. The RNA and protein was extracted at 2, 4, 6, and 8 hours. Fig 4 shows the percentage difference in incorporation of tracer into RNA and protein in ethylene-treated versus control explants. Enhancement of RNA synthesis occurs after an hour lag; the enhancement in protein synthesis occurs after a two-hour lag. These results indicate that stimulation of protein synthesis occurs only after a stimulation in RNA synthesis.

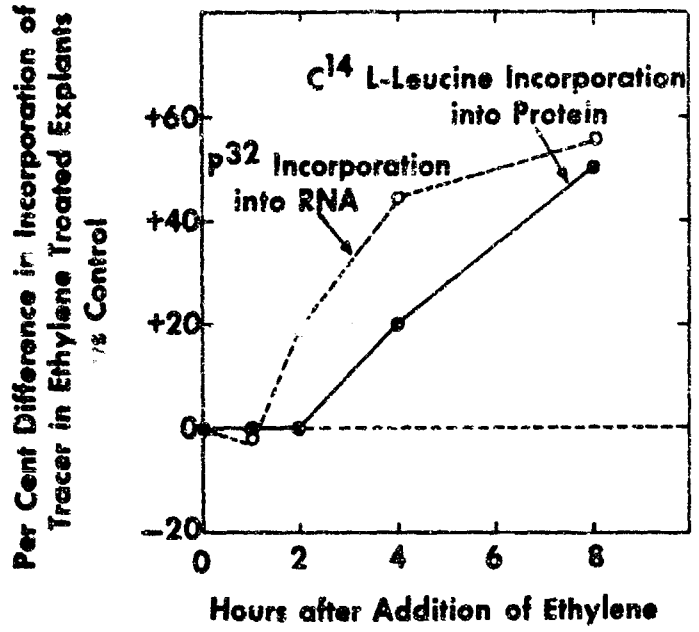

Figure 4. Time Course of Enhancement of P^{32} and C^{14} L-Leucine Incorporation into RNA and Proteins of Ethylene-Treated vs. Control Bean Explants.

E. EFFECT OF ETHYLENE ON JUVENILE AND SENESCENT EXPLANTS

In an earlier paper[1] we presented evidence that the stimulation of protein formation occured only in the separation layer of cotton and bean explants and not in the surrounding petiole or nodal tissue. Another way of demonstrating that ethylene action is specifically directed toward ethylene-sensitive tissue is to treat juvenile explants with the gas. Yamaguchi[12] and Abeles and Rubinstein[3] have shown that ethylene was without effect on abscission when juvenile explants were treated with gas. It follows that if the gas has no effect on abscission at this time, it should also have no effect on protein synthesis. Figure 5 shows that this is true; an ethylene-dependent enhancement of protein synthesis is absent in juvenile explants but readily observable in senescent explants.

F. EFFECT OF CARBON DIOXIDE ON ABSCISSION AND PROTEIN SYNTHESIS

CO_2 is known to block the effect of ethylene in accelerating abscission[12] and fruit ripening.[28] Burg and Burg[22] have shown with a Lineweaver and Burke kinetic analysis that CO_2 acts as a competitive inhibitor in the inhibition of pea stem growth by ethylene. Since CO_2 inhibits abscission, it also should inhibit the acceleration of protein synthesis by ethylene (i) if the assumption that the carbon dioxide occupies the same site as ethylene can be extrapolated from data on pea stem growth, and (ii) if the stimulation of protein synthesis is an integral part of abscission.

Data presented in Table 3 support this interpretation. This table shows that 15% CO_2 blocks abscission of bean explants and that the inhibiting effect of the CO_2 is partially relieved by the addition of 0.75 ppm ethylene. In another experiment using the same concentration of gas, CO_2 inhibited incorporation of leucine into protein by 7%, which might reflect the interaction of CO_2 with endogenous ethylene production. As usual, 0.75 ppm ethylene stimulated protein synthesis (by 30%) and this stimulation was almost completely overcome by 15% CO_2. Addition of ethylene and CO_2 simultaneously gave a rate of protein synthesis and abscission somewhat greater than that of the controls.

TABLE 3. INHIBITION OF EXPLANTS[a] ABSCISSION AND PROTEIN SYNTHESIS BY CARBON DIOXIDE

Treatment	% Abscission	CPM/mg Protein ± S.D.
Control	0	21,100 ± 400
15% CO_2	0	19,600 ± 700
0.75 ppm ethylene	100	27,200 ± 100
15% CO_2 + 0.75 ppm ethylene	80	21,600 ± 300

a. Explants were aged 22 hours at 25 C before C^{14} L-leucine in agar blocks was applied to the pulvinal stump, incubated in the above atmospheres for five hours, and then the protein was extracted by the TCA method.

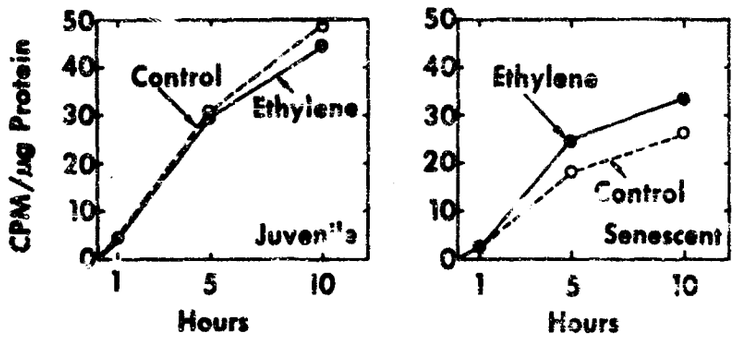

Figure 5. Effect of Ethylene on C^{14} L-Leucine Incorporation into Abscission-Zone Proteins of Juvenile and Senescent Explants.

G. NATURE OF PROTEINS FORMED IN PRESENCE OF ETHYLENE

We know that the ultimate effect of ethylene in abscission is due to a digestion of the intercellular material that normally holds the cells together.[23,24] Rubinstein and Leopold[25] have recently reviewed some of the enzymes that have been suggested as playing a role in this process. At this time, however, there is no agreement on the exact nature of these proteins. We have found that the protein labeled is soluble and is readily precipitated by low concentrations of ammonium sulfate (Table 4).

TABLE 4. FRACTIONATION OF ABSCISSION-ZONE PROTEINS BY AMMONIUM SULFATE[a/]

Fraction	Precipitant	Treatment	Protein, µg	CPM/mg Protein + S.D.	Stimulation, %
1	1.2 M NH_4SO_4	control	166	3,360 ± 590	67
		ethylene	184	5,600 ± 1,090	
2	1.8 M NH_4SO_4	control	480	4,430 ± 380	42
		ethylene	504	6,290 ± 1,000	
3	2.4 M NH_4SO_4	control	248	2,770 ± 100	21
		ethylene	232	3,360 ± 120	
4	5% final conc.	control	124	1,730 ± 140	17
	TCA	ethylene	114	2,020 ± 130	

a. Explants were aged for 20 hours at 25 C and then C^{14} L-leucine was applied to the pulvinal stump in agar blocks. Explants were then stored in either air or 4 ppm ethylene. Protein was extracted 8 hours later with Tris (10.4 g/liter), boric acid (5.5 g/liter), and disodium ethylenediaminetetraacetic acid (0.92 g/liter) pH 8.5 buffer. Protein precipitated by the ammonium sulfate was prepared for radioactivity measurements by the method of Key.[10]

IV. DISCUSSION

Ethylene appears to stimulate abscission of senescent explants only. Some of the factors that maintain juvenility of explants, measured as an insensitivity toward ethylene, include storage at low temperatures[12] and treatment with auxins,[3] cytokinins, and carbon dioxide. After the tissue has aged, carbon dioxide can still block ethylene action although auxins and cytokinins are without effect. This second effect of carbon dioxide may be similar to the competitive inhibitor effect known for the inhibition of pea stem growth by ethylene.[22] This twofold action of carbon dioxide on abscission, first retarding senescence and second blocking ethylene action, was previously recognized by Yamaguchi.[12] The senescence-retarding abilities of cytokinins measured by other parameters such as maintenance of the chlorophyll content of leaves[28] are also well known. Some of the factors, i.e., shortened photoperiod, trauma, and general nutrition known to initiate senescence in leaves are discussed in Addicott's[27] comprehensive review of this subject.

Once the abscission zone becomes receptive, the action of ethylene appears to be similar to that of other plant and animal hormones. Although the idea of a gaseous hormone is not new[28] it has never achieved the popularlity accorded the less ephemeral ones such as auxin, gibberellins and cytokinins. Nevertheless, ethylene is known to be evolved from fruits, flowers, leaves, stems, roots, tubers, and seeds from a large variety of plants[29] and, characteristic of hormones, is active in small amounts (ca. 1 ppm) for most of its effects. Since the gas is produced in all parts of the plant, translocation from the site of production to the site of action does not seem to be a problem.

The observation that ethylene action is blocked by actinomycin D and cycloheximide is evidence in favor of an effector role for ethylene. The fact that the inhibiting effect of cycloheximide persisted longer than that of actinomycin D after the addition of ethylene is evidence that the mode of action of these compounds in explants is similar to that ascribed to them from studies of other systems, that is, the inhibiting effect of cycloheximide lasts longer than that of actinomycin D because it acts at a later point in protein synthesis.

Large doses of X-irradiation also blocked the effect of ethylene. Although no direct evidence is offered, we assumed that the effect of the radiation was preferentially on the nucleic acid - dependent part of protein synthesis and that proteins were more resistant to the action of this high-energy radiation. This assumption was supported by the fact that X-irradiation was most effective prior to the addition of ethylene and that after polypeptide synthesis had taken place, its effectiveness was lost.

When we examined the effect of ethylene on RNA and protein synthesis, we found that results with explant tissue agreed with what is well known for polypeptide synthesis in other organisms; that is, a short lag period occurs after the addition of ethylene before the synthesis of RNA begins followed by another lag period and the stimulation of protein synthesis. As anticipated, actinomycin D blocked the stimulation of RNA synthesis by ethylene. Additional support for the idea that ethylene-dependent protein synthesis is an integral part of abscission comes from the experiments that show that carbon dioxide blocked both abscission and protein synthesis. When the inhibiting effect of the carbon dioxide was overcome by additonal amounts of ethylene, protein synthesis also proceeded.

In this paper we offer evidence in favor of the hypothesis that ethylene is a plant hormone by presenting data showing that ethylene is capable of acting as an effector substance. Another possible interpretation of these data includes the idea that ethylene treatment causes another substance to be an effector, either through synthesis or release by alteration of celluler compartmentalization. This idea is not advanced to avoid the risk of speculation but represents a real problem in studies with plant hormones. It is well known that IAA[7] can stimulate RNA synthesis. It is also known that IAA can stimulate ethylene synthesis, and interestingly enough the effect of this IAA can be blocked by actinomycin D and puromycin.[30] Since we show that ethylene is capable of stimulating RNA synthesis under certain circumstances, how can we be sure that auxin effects are not ethylene effects?

Burg and Burg[31] have shown that the inhibiting effect of high concentrations of IAA on pea stem growth is probably due to enhanced ethylene production. Other effects of growth regulators such as epinasty, guttation, release of dormancy, root initiation, floral initiation of the pineapple, fruit ripening, and intumescence formation that are known to be similarly influenced by ethylene could be profitably re-examined (see Abeles and Rubinstein[3] for appropriate references).

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13. ABSTRACT

Ethylene stimulated RNA and protein synthesis in bean (Phaseolus vulgaris L. var. Red Kidney) abscission zone explants prior to abscission. The effect of ethylene on RNA synthesis and abscission was blocked by actinomycin D. Carbon dioxide, which inhibits the effect of ethylene on abscission, also inhibited the influence of ethylene on protein syntheses. An aging period appears to be essential before bean explants respond to ethylene. Kinetins, which retard senescence, will also block the action of ethylene. Stimulation of protein synthesis by ethylene occurred only in receptive or senescent explants. Treatment of juvenile explants with ethylene, which has no effect on abscission, also has no effect on protein synthesis. Evidence in favor of a hormonal role for ethylen during abscission is discussed.

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